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iCell[®] GlutaNeurons

User's Guide



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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell GlutaNeurons as described herein. The only warranties provided to you are included in the Limited Warranty enclosed with this guide. You assume all risk in connection with your use of iCell GlutaNeurons.

Conditions of Use

iCell GlutaNeurons are for life science research use only and subject to the use restrictions contained in Appendix A. You are responsible for understanding and performing the protocols described within this guide. CDI does not guarantee any results you may achieve. These protocols are provided as CDI’s recommendations based on its use and experience with iCell GlutaNeurons.

Origin

iCell GlutaNeurons are manufactured in the United States of America.

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Revision History

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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® GlutaNeurons User's Guide before handling or using iCell GlutaNeurons.
- iCell GlutaNeurons are for life science research use only. See Appendix A for more information and other restrictions.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell GlutaNeurons are frozen, is available online at www.cellulardynamics.com/lit/ or on request from Cellular Dynamics International. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell GlutaNeurons.

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell GlutaNeurons are a highly pure population of human glutamatergic neurons derived from induced pluripotent stem (iPS) cells using CDI's proprietary differentiation and purification protocols. iCell GlutaNeurons are a mixture of post-mitotic neuronal subtypes, composed predominantly of cortical glutamatergic neurons with typical physiological characteristics and functional neuronal networks. These cells provide a reliable source of human neurons suitable for use in targeted drug discovery, toxicity testing, and other life science research.

When handled and maintained as recommended in this User's Guide, iCell GlutaNeurons quickly assume a typical neuronal morphology with branching neurites (Figure 1). In addition, these cells display a stable adherent single-cell morphology and remain viable for an extended culture period (≥ 14 days) making them amenable to a variety of electrophysiology, neurotoxicity, and neurotransmission assays.

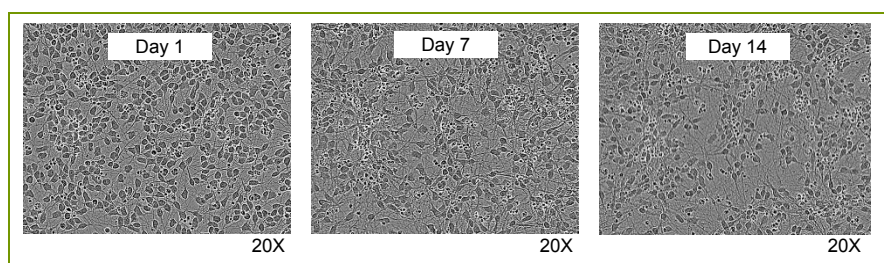


Figure 1: iCell GlutaNeurons Exhibit Typical Neuronal Morphology

These images show iCell GlutaNeurons at days 1, 7, and 14 post-plating. Re-animated iCell GlutaNeurons develop branched networks within 2 - 3 days and remain viable and adherent for an extended period in culture (≥ 14 days).

Components Supplied by Cellular Dynamics

Notes

Item	Catalog Number
iCell GlutaNeurons ¹	GNC-301-030-000.5 (0.5 unit) GNC-301-030-001 (1 unit)
iCell DopaNeurons Medium Supplement ¹	DNM-301-031-001
iCell Nervous System Supplement ¹	NSS-301-031-001
iCell GlutaNeurons User's Guide ¹	
Certificate of Analysis ²	
Certificate of Origin If required for shipping purposes	
1 Safety Data Sheets and User's Guide available online at www.cellulardynamics.com/lit/	
2 Available online at www.cellulardynamics.com/coa/	

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter*	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
0.22 µm Sterile Filter Unit	Multiple Vendors	
50 ml Centrifuge Tubes	Multiple Vendors	
24-well Cell Culture Plates	Nunc	142475
6-well Cell Culture Plates	Nunc	140675
96-well Cell Culture Plates	Corning	3603
BrainPhys Neuronal Medium	STEMCELL Technologies	05790
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS)	Thermo Fisher Scientific	14190
Growth Factor Reduced Matrigel Matrix (Matrigel)	Corning	354230
Laminin	Sigma-Aldrich	L2020
N-2 Supplement, 100X	Thermo Fisher Scientific	17502-048
Penicillin-streptomycin, 100X	Thermo Fisher Scientific	15140-122
Pipettes	Multiple Vendors	
Poly-L-Ornithine (PLO)	Sigma-Aldrich	P4957
Trypan Blue	Thermo Fisher Scientific	15250

* Ensure the automated cell counter is appropriately calibrated before use.

Technical Support and Training

CDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. In addition, in-lab training may be available upon request.

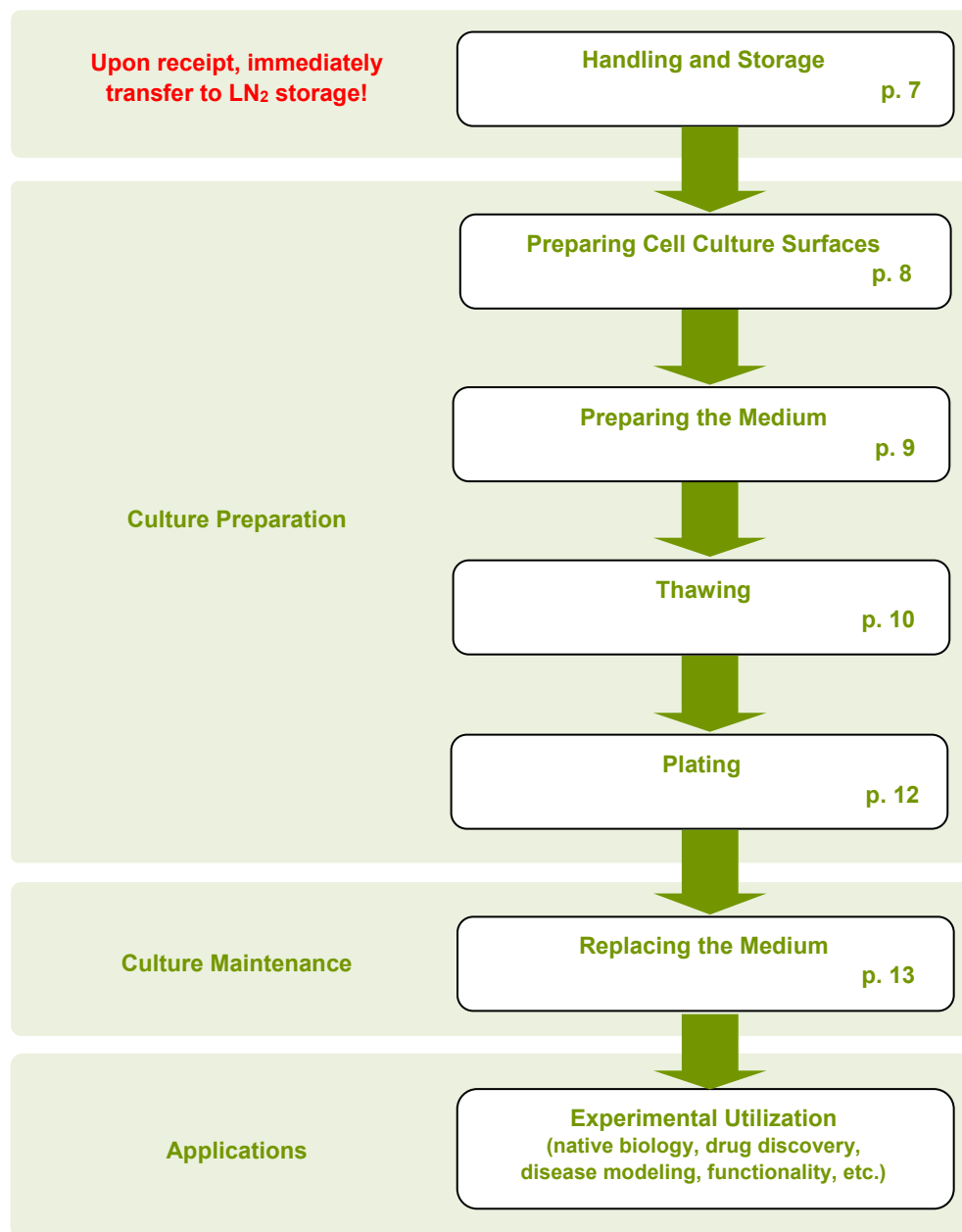
Telephone (877) 320-6688 (US toll-free) / (608) 310-5100 x5
Monday - Friday, 8:30 am - 5:00 pm US Central Time

Fax (608) 310-5101

Email support@cellulardynamics.com

Workflow Diagram

Notes



Chapter 2. Handling and Storage

Handling iCell GlutaNeurons

iCell GlutaNeurons are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer iCell GlutaNeurons to the vapor phase of a liquid nitrogen storage dewar.



It is critical to maintain cryopreserved iCell GlutaNeurons at a stable temperature. Minimize exposure of cryopreserved iCell GlutaNeurons to ambient temperature when transferring vials to liquid nitrogen storage.

Handling iCell Neuronal Supplements

iCell GlutaNeurons are shipped with two additional components: iCell DopaNeurons Medium Supplement and iCell Nervous System Supplement. iCell DopaNeurons Medium Supplement and iCell Nervous System Supplement are shipped frozen on dry ice. Upon receipt, store these supplements at -20°C until ready for use.

Chapter 3. Preparing Cell Culture Surfaces

iCell GlutaNeurons will plate and function on a freshly prepared plate with a base layer of poly-L-ornithine (PLO) and a top coating of Matrigel, which are recommended to promote iCell GlutaNeurons attachment, viability, and function.

Prepare plating surfaces before thawing iCell GlutaNeurons.

1. Use the volumes specified in the table below in the following coating procedure. Scale volumes appropriately for other vessel formats.

Culture Vessel	Volume of 0.01% PLO Solution (ml)	Volume of D-PBS Rinse (ml)	Volume of 0.028 mg/ml Matrigel Solution (ml)
6-well Cell Culture Plate	1	2	1
24-well Cell Culture Plate	0.6	1.2	0.6
96-well Cell Culture Plate	0.1	0.2	0.1

Table 1: Summary of Useful Volumes

All volumes are per well.

2. Add 0.01% PLO solution to each well of the vessel(s).
3. Incubate the vessel(s) at room temperature for at least 1 hour.
4. Prepare the complete BrainPhys medium according to the instructions in Chapter 4, Preparing the Medium. Dilute 0.3 mg of Matrigel in 10.5 ml of ice-cold complete BrainPhys medium to a final concentration of 0.028 mg/ml.
5. After incubation of the vessel(s), completely aspirate the PLO solution from each well. Rinse each well 3 times with D-PBS and aspirate completely.

Note: *Rinsing each well thoroughly is critical to avoid PLO-induced cell toxicity.*

6. Add 0.028 mg/ml Matrigel solution to each well and incubate the vessel(s) in a 37°C cell culture incubator for at least 1 hour.

Note: *Alternatively, add the Matrigel solution to each well, wrap the vessel(s) in plastic paraffin film, and store overnight at 4°C. Equilibrate the vessel(s) in a 37°C cell culture incubator before use.*

7. Aspirate the Matrigel solution immediately before the addition of the cell suspension.



Do not allow the Matrigel-coated surface to dry. Drying of the culture surface can lead to cell clumping and migration.

Chapter 4. Preparing the Medium

iCell GlutaNeurons are cultured in complete BrainPhys medium comprised of the BrainPhys Neuronal Medium, iCell DopaNeurons Medium Supplement, iCell Nervous System Supplement, N-2 supplement, laminin, and penicillin-streptomycin. The complete BrainPhys medium is serum-free and has been specially formulated to maintain the health and function of iCell GlutaNeurons while limiting the proliferation of progenitor or non-neuronal cells. iCell GlutaNeurons can be maintained in culture for at least 2 weeks in this medium without appreciable loss of viability or purity.

Complete BrainPhys Medium Components	Volume	Final Concentration
BrainPhys Neuronal Medium	95 ml	Not Applicable
iCell DopaNeurons Medium Supplement	2 ml	Not Applicable
iCell Nervous System Supplement	1 ml	Not Applicable
N-2 Supplement	1 ml	Not Applicable
Laminin	100 μ l	1 μ g/ml
Penicillin-streptomycin	1 ml	Not Applicable

Table 2: Volumes for Complete BrainPhys Medium Preparation

1. Thaw iCell DopaNeurons Medium Supplement, iCell Nervous System Supplement, N-2 supplement, and laminin at room temperature on the day of medium preparation.



Do not thaw the supplements and laminin in a 37°C water bath.

2. Spray all medium components with 70% ethanol and place in a biological safety cabinet.
3. Using sterile technique, add the entire contents of iCell DopaNeurons Medium Supplement (~2 ml), iCell Nervous System Supplement (~1 ml), N-2 supplement (1 ml), laminin (100 μ l) and penicillin-streptomycin (1 ml) to the BrainPhys Neuronal Medium (95 ml) to make the complete BrainPhys medium. Filter the complete BrainPhys medium through a 0.22 μ m sterile filter unit.
4. Store the complete BrainPhys medium at 4°C, protected from light, for up to 2 weeks.

Note: CDI recommends using room temperature complete BrainPhys medium to thaw iCell GlutaNeurons.

Note: Freeze remaining N-2 supplement in 1 ml aliquots. Do not refreeze the other individual medium components or complete BrainPhys medium.

Chapter 5. Thawing iCell GlutaNeurons

Maintain iCell GlutaNeurons in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell GlutaNeurons viability and performance.

Note: Thaw no more than 1 vial of iCell GlutaNeurons at one time.

1. Equilibrate the complete BrainPhys medium at room temperature before thawing iCell GlutaNeurons.
2. Remove the iCell GlutaNeurons cryovial from the liquid nitrogen storage tank.

Note: If necessary, place the cryovial on dry ice for up to 10 minutes before thawing.

3. Immerse the cryovial in a 37°C water bath for **exactly 2 minutes** (avoid submerging the cap) holding the tube stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.



Precise timing is critical to maximizing viable cell recovery.

4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iCell GlutaNeurons cryovial contents to a sterile 50 ml centrifuge tube using a 1 ml pipettor.

Note: Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase neuron viability.



Avoid repeated pipetting of the thawed iCell GlutaNeurons cell suspension.

6. Rinse the empty iCell GlutaNeurons cryovial with 1 ml of room temperature complete BrainPhys medium to recover any residual cells from the vial. Transfer the 1 ml of the complete BrainPhys medium rinse from the cryovial drop-wise (~1 drop/sec) to the 50 ml centrifuge tube containing the iCell GlutaNeurons cell suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize the osmotic shock on the thawed cells.



Drop-wise addition of the complete BrainPhys medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and attachment.

7. Slowly add 8 ml of room temperature complete BrainPhys medium to the 50 ml centrifuge tube drop-wise (~1 drops/sec) while gently swirling.



It is critical to add the 8 ml of complete BrainPhys medium slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.

Notes

8. Centrifuge the cell suspension at 400 x g at room temperature for 5 minutes.
9. Carefully aspirate the supernatant, leaving ≥ 0.5 ml in the centrifuge tube.



Leaving <0.5 ml of medium increases the risk of aspirating a portion of the cell pellet.

10. Gently resuspend the cell pellet in an appropriate volume (e.g. 2 - 3 ml) of the complete BrainPhys medium by pipetting up and down 2 - 3 times.

Chapter 6. Plating iCell GlutaNeurons

The recommended plating density for iCell GlutaNeurons is $1.9 - 2.5 \times 10^5$ viable cells/cm² ($6 - 8 \times 10^4$ cells/well for a 96-well plate). See Figure 2 for images showing cells plated at alternative plating densities.

1. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
2. Dilute the cell suspension using room temperature complete BrainPhys medium to obtain a desired cell plating density.
3. Aspirate the Matrigel solution from the pre-coated cell culture plates and immediately dispense the cell suspension.
4. Culture iCell GlutaNeurons in a cell culture incubator at 37°C, 5% CO₂.

Expected Cell Density

iCell GlutaNeurons can be plated at various densities to accommodate different applications (Figure 2). However, 2.5×10^5 viable cells/cm² is the recommended density for most applications. The following table provides the desired cell number and plating volume for several common cell culture vessels when plating at a density of 2.5×10^5 viable cells/cm².

Culture Vessel	Surface Area (cm ²)	Plating Volume (ml)	Cell Number (2.5×10^5 cells/cm ²)
6-well Cell Culture Plate	9.6	2	2.4×10^6
24-well Cell Culture Plate	1.9	0.6	4.8×10^5
96-well Cell Culture Plate	0.32	0.1	8.0×10^4

Table 2: Summary of Recommended Volumes and Measures

All volumes and measures are *per well*.

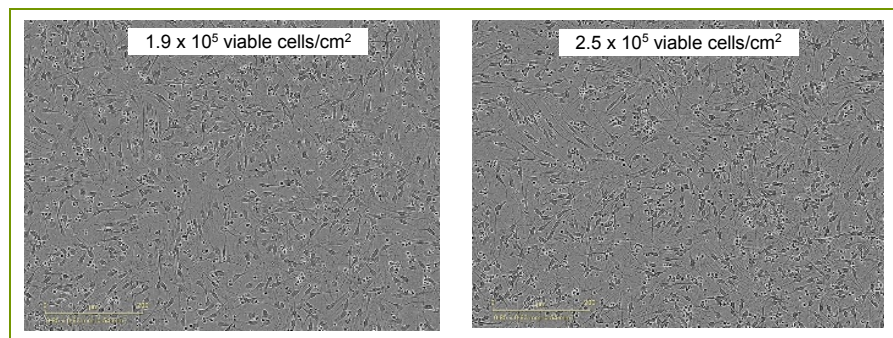


Figure 2: iCell GlutaNeurons Plated at Two Densities

These images show iCell GlutaNeurons at day 7 post-plating when plated at 1.9×10^5 and 2.5×10^5 viable cells/cm² into a PLO/Matrigel-coated 96-well cell culture plate.

Chapter 7. Maintaining iCell GlutaNeurons

When plated and maintained in complete BrainPhys medium, iCell GlutaNeurons are able to persist in culture while retaining a high level of purity for at least 2 weeks post-plating.



The complete BrainPhys medium is stable for 2 weeks when stored at 4°C.

1. Immediately before use, equilibrate the complete BrainPhys medium to room temperature for at least 30 minutes.

Note: Do not equilibrate the complete BrainPhys medium to 37°C.



Repeated warming of the complete BrainPhys medium may decrease stability.

2. The day after plating iCell GlutaNeurons in a 96-well plate, add 100 µl of the complete BrainPhys medium to each well (200 µl total volume). For larger well formats, perform a 100% medium exchange.
3. For cells plated in a 96-well plate, perform a 50% medium exchange every other day. For larger well formats, perform a 100% medium exchange every other day.



It is critical to gently dispense the complete BrainPhys medium to the side of the well to avoid cell detachment.

4. Culture iCell GlutaNeurons in a cell culture incubator at 37°C, 5% CO₂.

Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

A. OWNERSHIP. The Products are covered by pending patents and patents: cellulardynamics.com/about-us/patents/. Customer has a limited license to use the Products for internal research purposes for the sole benefit of the Customer, subject to the use restrictions included in subsection B of this Appendix A. Customer acknowledges and agrees that the receipt or purchase of the Products by Customer shall not be construed as a transfer of any title or the grant of any rights in or to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. In particular, no right or license to make, have made, offer to sell, or sell the Products, to modify or reproduce the Product or any part thereof, or to use the Products in combination with any other product(s), except product(s) provided or expressly licensed to Customer by Cellular Dynamics for such use, is implied or conveyed by the sale or transfer of Products to Customer.

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Appendix B. Limited Warranty

A. During the Warranty Period (as defined below) and subject to subsection F of this Appendix B. Cellular Dynamics warrants that its Products conform to the specifications contained in the Certificate of Analysis for the Product shipped to Customer. Customer's sole and exclusive remedy (and Cellular Dynamics' sole and exclusive liability) with respect to any defective Products shall be replacement of the defective Products by Cellular Dynamics pursuant to this Appendix B.

B. Under no circumstances shall Cellular Dynamics' liability to Customer exceed the amount paid by Customer for the Products to Cellular Dynamics. Cellular Dynamics will bear all reasonable shipping costs if the Products are replaced pursuant to this warranty. For clarity, this warranty automatically shall be void, and any claims under it invalid, (i) if Customer's use of the Products is other than solely in accordance with this User's Guide and Cellular Dynamics' Terms and Conditions (or such other written agreement between Cellular Dynamics and Customer under which the Products are sold or transferred to Customer) or for a purpose or in a manner other than that for which the Products were designed; or (ii) if Customer fails to follow this User's Guide for the use, storage, and handling of the Products however such failure is caused; or (iii) if Customer fails to comply with any of the provisions of Appendix A in this User's Guide; or (iv) if there is any abuse, other misuse or neglect of the Products by Customer or to the extent of any damage or loss of the Products by events or occurrences beyond a person's (e.g., Cellular Dynamics') control including without

limitation, accident, fire, vandalism and natural disasters (acts of God). This warranty applies only to Customer and not to third parties. This warranty is not assignable.

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D. Within five (5) business days of thawing the Product but prior to the expiration date of the Product as listed on the Certificate of Analysis and/or Product's label (the "Warranty Period"), Customer must notify Cellular Dynamics in writing of any nonconformity of the Products, describing the nonconformity in detail. Customer's failure to properly notify Cellular Dynamics in the Warranty Period voids the limited warranty set forth above in this Appendix B.

E. Customers who believe they have a warranty claim should call Cellular Dynamics' Technical Support line at (608) 310-5100 ext. 5 or email at support@cellulardynamics.com to request a replacement Product based on a breach of the limited warranty set forth above in this Appendix B. Any action by Customer for Cellular Dynamics' breach of this limited warranty, for which Customer has given timely and proper notice of such breach during the Warranty Period and otherwise in accordance with this Appendix B, must be commenced by Customer within 18 months following the date of such breach.

F. Cellular Dynamics makes no warranty of any kind or nature, neither express nor implied, for any product sold together with, or as a part of, the Products (e.g., an accessory accompanying a Product or a discrete component part of a Product that is a kit) that is not manufactured by Cellular Dynamics. Any such accessory to or part of the Products shall have the warranty, if any, that is offered and granted (and, for clarity, extended by its terms to Customer) by the manufacturer of such other accessory or component product accessories.

G. Customer acknowledges and agrees that Cellular Dynamics may fill Customer's order with any number of units of Products. Such units may be more units than Customer ordered. Customer will not be charged extra for any adjustments made by Cellular Dynamics. The number of cells in a unit is determined by the Product's Certificate of Analysis. The number of cells that are contained in a unit accounts for both viability and plating efficiency percentages. Because this may vary from lot to lot, Cellular Dynamics reserves the right to fill the order with that number of units which is sufficient to fill Customer's order and such adjustments shall not constitute a breach of the limited warranty set forth herein.

Appendix C. Limited Liability

TO THE FULLEST EXTENT PERMITTED UNDER APPLICABLE LAW, CELLULAR DYNAMICS SHALL NOT HAVE ANY LIABILITY FOR INCIDENTAL, COMPENSATORY, PUNITIVE, CONSEQUENTIAL, INDIRECT, SPECIAL OR OTHER SIMILAR DAMAGES, HOWEVER CAUSED AND REGARDLESS OF FORM OF ACTION WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT PRODUCT LIABILITY OR OTHERWISE, EVEN IF CELLULAR DYNAMICS HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. NOTWITHSTANDING ANY OTHER TERM OR IMPLICATION TO THE CONTRARY, UNDER NO CIRCUMSTANCES SHALL CELLULAR DYNAMICS' LIABILITY TO CUSTOMER EXCEED THE AMOUNT PAID BY CUSTOMER FOR THE PRODUCTS TO CELLULAR DYNAMICS.

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